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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/761,893	01/17/2001	Shih-Chieh Hung	11709-003001	6011
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Shih-Chieh Hung Dept. of Orthop. and Traumatology, Vet. General 201, Sec. 2, Shih-pai Road Hospital-Taipci Taipei, 11217 TAIWAN			EXAMINER DUNSTON, JENNIFER ANN	
			ART UNIT	PAPER NUMBER
			1636	
			MAIL DATE	DELIVERY MODE
			08/13/2010	PAPER

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

### Office Action Summary

**Application No.**

09/761,893

**Applicant(s)**

HUNG ET AL.

**Examiner**

Jennifer Dunston

**Art Unit**

1636

**Period for Reply** -- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

- 1) ☒ Responsive to communication(s) filed on 22 February 2010 and 01 June 2010.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

- 4) ☒ Claim(s) 1, 4, 6, 9-20 and 34-38 is/are pending in the application.
- 4a) Of the above claim(s) 12-20 is/are withdrawn from consideration.
- 5) ☐ Claim(s) \_\_\_\_\_ is/are allowed.
- 6) ☒ Claim(s) 1, 4, 6, 9-11 and 34-38 is/are rejected.
- 7) ☐ Claim(s) \_\_\_\_\_ is/are objected to.
- 8) ☐ Claim(s) \_\_\_\_\_ are subject to restriction and/or election requirement.

**Application Papers**

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☒ The drawing(s) filed on 17 January 2001 is/are: a) ☒ accepted or b) ☐ objected to by the Examiner.
- Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
- Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

- 12) ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☒ All b) ☐ Some \* c) ☐ None of:
1. ☒ Certified copies of the priority documents have been received.
  2. ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
  3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

\* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

- 1) ☒ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☐ Information Disclosure Statement(s) (PTO/SF-08)
- 4) ☐ Interview Summary (PTO-413)
- 5) ☐ Notice of Informal Patent Application
- 6) ☐ Other: \_\_\_\_\_

### **DETAILED ACTION**

This action is in response to the amendment, filed 6/1/2010, in which claim 33 was canceled, and claims 1 and 38 were amended. Claims 1, 4, 6, 9-20 and 34-38 are pending.

Applicant's arguments have been thoroughly reviewed, but are not persuasive for the reasons that follow. Any rejections and objections not reiterated in this action have been withdrawn. **This action is FINAL.**

#### ***Election/Restrictions***

Applicant elected Group I without traverse in the reply filed on 9/4/2001.

Claims 12-20 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 9/4/2001.

Currently, claims 1, 4, 6, 9-11 and 34-38 are under consideration.

#### ***Response to Arguments - Claim Objections***

The previous objection of claim 38 has been withdrawn in view of Applicant's amendment to the claim in the reply filed 6/1/2010.

#### ***Claim Rejections - 35 USC § 112***

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1, 4, 6, 9-11 and 34-38 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. This rejection was made in the Office action mailed 1/22/2010 and has been rewritten to address the amendments to the claims in the reply filed 6/1/2010.

Enablement is considered in view of the Wands factors (MPEP 2164.01(A)). These include: nature of the invention, breadth of the claims, guidance of the specification, the existence of working examples, state of the art, predictability of the art and the amount of experimentation necessary. All of the Wands factors have been considered with regard to the instant claims, with the most relevant factors discussed below.

*Nature of the invention:* The claims are drawn to a method for isolating mesenchymal stem cells (MSCs) from bone marrow aspirate, and the claims explicitly require the separation of MSCs from hematopoietic stem cells (HSCs).

Independent claim 1 comprises the following method steps: (a) providing a cell mixture comprising MSCs and other cells; (b) seeding and culturing the cell mixture in a culture device comprising an upper plate with pores and a lower plate base, said upper plate made of MSC adhering material and has pores of about 0.4 to 40 microns in diameter, where MSCs adhere and are cultured, and the lower plate base, where the other small-sized HSCs adhere following passing through the pores in the upper plate, said culturing with medium containing factors that stimulate MSC growth without differentiation and allows for the selective adherence of only

MSCs to the upper plate surface; and (c) removing non-adherent cells on the upper plate by changing medium. Dependent claims 4, 6, 9 and 10 further limit the characteristics of the MSC. Claims 11 and 35-38 further limit the culturing conditions. Claim 11 is drawn to the use of culture medium comprising 10% fetal bovine serum-supplemented Dulbecco's modified Eagle's medium containing 1g/L of glucose. Dependent claim 34 limits the mesenchymal stem cell adhering material to plastic.

The claimed invention seeks to exploit the biological and physical characteristics of MSCs to provide an isolated population of MSCs. With regard to the biological properties, the claim requires the selective adherence of MSCs to the top plate. With regard to physical properties, the claim requires size selection of MSCs. Specifically, the upper plate with pores must separate MSCs from HSCs. The upper plate must retain the MSCs while allowing HSCs to pass through the pores.

*Breadth of the claims:* The claims broadly encompass the use of a culture device comprising an upper plate that contains pores of any size. The complex nature of the subject matter of this invention is greatly exacerbated by the breadth of the claims.

*Guidance of the specification and existence of working examples:* The specification envisions using the physical and biological characteristics of MSCs to isolate MSCs from a cell mixture. Specifically, the specification envisions using a difference in cell size and a difference in adherence to isolate MSCs (e.g., page 7, lines 10-17). The specification envisions using a plate with pores, where the pore size is defined functionally as being "sufficient for separating mesenchymal stem cells from other cells (e.g. haematopoietic stem cells)" (page 7, lines 24-27).

The specification asserts that a preferable pore size is from about 0.4 to 40 microns in diameter (e.g., page 7, lines 27-29; page 8, lines 29-30).

A working example of the claimed method is presented on page 11-12 of the specification; however, the working example **does not disclose the pore size** of the upper plate. Percoll fractionated or un-fractionated bone marrow cells in DMEM-LG with 10% FBS and antibiotics were seeded into a culture device at a density of  $10^6/\text{cm}^2$  (page 11, lines 20-25). The design specifics of the culture device used in the working example are not disclosed. The specification notes that HSCs and non-adherent cells were removed with changes in medium (page 12, lines 4-6; page 14, lines 18-20). The cells retained by the upper plate were analyzed and found to have characteristics consistent with a MSC phenotype (e.g., page 12, line 16 to page 15, line 28). At page 14, the specification discusses the characteristics of the cells that are collected on the bottom plate (i.e., the cells that pass through the undefined pores). The cells were described as having a small size, polygonal shape, and little renewal capacity (page 14, lines 7-9). The specification states that the cells "were believed to be haematopoietic cells" (page 14, line 10).

The specification does not provide any characterization of the cells that are collected on the lower plate and are "believed to be haematopoietic cells." The cell surface markers of the cells were not analyzed (e.g., CD34). The ability of the cells to differentiate along the hematopoietic lineage or perform hematopoietic reconstitution is not disclosed.

*Predictability and state of the art:* The state of the art with regard to the separation of MSCs and HSCs based upon size selection through pores was underdeveloped at the time the invention was made. Prockop et al (US Patent No. 7,374,937 B1, effective date March 14, 2000,

cited in a prior action) teaches the isolation of MSCs from bone marrow aspirates obtained from the iliac crest of normal human donors (e.g., column 24, line 24 to column 35, line 5). The non-adherent cells were removed, and the adherent cells were harvested (e.g., paragraph bridging columns 34-35). Prockop et al assayed the MSCs for size and granularity by forward light and side light scattering by FACS (e.g., column 36, lines 9-41). When the MSCs were plated at a low density, a population of large cells with a medium content of granules was observed (mature MSCs or mMSCs) along with two populations of small cells: (1) small and agranular cells referred to as recycling stem cells-1 (RS-1); and (2) small and granular cells referred to as recycling stem cells-2 (RS-2) (e.g., column 36, lines 31-34). Prockop et al teach that a polycarbonate membrane with a pore size of 10 micrometers separates the mMSCs from the RS cells (e.g., column 39, line 60 to column 40, line 42). Prockop et al teach that the small cells that pass through the pores are CD34-negative (e.g., Figure 26). The present specification teaches that HSCs obtained from bone marrow are CD34-positive (e.g., page 14, lines 26-27). Thus, the small cells separated from the MSC of bone marrow aspirate using a 10 micron filter do not appear to be HSCs. Further evidence that the small cells are not HSCs is provided by the post filing art. Colter et al teach that the RS cells are capable of adopting the same cell fates as MSCs in that they are capable of differentiating into osteoblasts, adipocytes and cartilage (Colter et al. Proceedings of the National Academy of Sciences, USA, Vol. 98, No. 14, pages 7841-7845, July 2001; e.g., paragraph bridging pages 7843-7844). Accordingly, it would be unpredictable to use a filter containing pores of 10 micrometers to separate MSCs from HSCs of bone marrow aspirate.

Applicant's own post-filing art teaches that MSCs were isolated from human bone marrow aspirates by the use of a "unique method that included a specially designed culture device, which was a plastic culture dish comprising a plate with 3- $\mu$ m pores to sieve out MSCs from bone marrow aspirates" (Hung et al. Stem Cells, Vol. 20, pages 249-258, 2002; e.g., page 250, right column). The specific culture device used was a 10-cm plastic culture dish comprising a plate with 3- $\mu$ m pores sold as a Transwell® device by Corning Inc. (e.g., page 251, paragraph bridging columns). The features of this device, such as the 3- $\mu$ m pore size, are not taught by the present specification.

The post filing art teaches that the cells collected on the lower plate base have never been characterized (Zuba-Surma et al. Cytometry Part A, Vol. 75A, pages 4-13, 2009; e.g., page 11, right column, last full paragraph). Given the lack of characterization of the small cells, and the evidence provided by Prockop et al and Colter et al that other small cells present in bone marrow are not HSCs, it would be unpredictable to separate MSCs from HSCs using a device with any size pores, or pores ranging from 0.4-40 microns.

*Amount of experimentation necessary:* The quantity of experimentation needed to carry out the claimed invention is large. One would be required to determine the pore sizes that provide separation of MSCs from HSCs obtained from bone marrow aspirate. The prior art teaches that pores of 10  $\mu$ m do not separate MSCs from HSCs; rather, pores of 10  $\mu$ m separate large MSCs from small MSCs (RS cells). Furthermore, the specification does not teach the pore sized used in the working example. Without the guidance of the post filing art, it would require a large amount of experimentation to determine the pore size that provides the result obtained in the specification. The post-filing art teaches a pore size of 3  $\mu$ m was used, and the specification

discloses any pore size, or preferably about 0.4 to 40  $\mu\text{m}$ . Even if one were to use a pore size of 3  $\mu\text{m}$ , there is no evidence on the record that the small cells obtained on the lower plate are HSCs. To determine if the cells are HSCs, one would be required to determine the cell surface markers and test the ability of the cells to differentiate along the hematopoietic lineage or perform hematopoietic reconstitution. The outcome of any experiment relying upon the guidance of the specification and prior art is unpredictable, and the type of experimentation required is not routine in the art.

In view of the breadth of the claims and the lack of guidance provided by the specification as well as the unpredictability of the art, the skilled artisan would have required an undue amount of experimentation to make and/or use the claimed invention. Therefore, claims 1, 4, 6, 9-11 and 34-38 are not considered to be enabled by the instant specification.

***Response to Arguments - 35 USC § 112***

The rejection of claim 33 under 35 U.S.C. 112, first paragraph is moot in view of Applicant's cancellation of the claim in the reply filed 6/1/2010.

With respect to the rejection of claims 1, 4, 6, 9-11 and 34-38 under 35 U.S.C. 112, first paragraph, Applicant's arguments filed 2/22/2010 have been fully considered but they are not persuasive.

At paragraph 2, the response states that devices with pore sizes of 0.4, 3, 5, 8 and 11  $\mu\text{m}$  were used and found to have worked when separating mesenchymal stem cells (MSCs) from other cells.

This argument is not found persuasive, because it is not supported by evidence presented in the form of a declaration. Furthermore, the claims require the porous plate to support mesenchymal stem cells while allowing haematopoietic cells to pass through the pores to a lower plate base. While filters containing pores of 0.4, 3, 5, 8 and 11  $\mu\text{m}$  may all support the attachment and culture of mesenchymal stem cells. The response does not state or provide evidence that haematopoietic cells passed through the pores to the lower plate base.

At paragraph 3, the response notes that Prockop used a frozen stock of MSCs which did not contain haematopoietic stem cells (HSCs). Further, the response notes that SP cells are CD34-negative but are precursors of CD34-positive HSCs. The response notes that the HSCs that pass through may be CD34-positive or CD34-negative.

The Examiner agrees that Prockop could not show migration of HSCs because they had been removed during prior culturing steps. However, the identity of the cells that pass through a 0.4 to 40  $\mu\text{m}$  have not been confirmed to be HSCs as required by the claim (see lines 9-10 of claim 1).

At paragraph 3, numerous non-patent literature references are cited to provide evidence that Transwell® dishes are used for CD34<sup>+</sup> or other cell migration. In other words, the references are cited to show that HSCs could pass through the claimed pore sizes, as required by the claims.

The references and passages cited in the response are not sufficient to overcome the rejection of record, because the evidence is not commensurate in scope with the claimed invention. While Applicant's reasoning that MSCs can have a diameter of 50  $\mu\text{m}$  or larger (paragraph 1) would enable a maximum pore size of 40  $\mu\text{m}$ , the evidence presented does not provide enablement for the smaller diameters of the claims. The smallest diameter that allows

the passage of cells appears to be 3  $\mu\text{m}$ . Applicant cites (Taichman et al. Blood, Vol. 89, No. 4, pages 1165-1172, February 1997) as evidence that a 0.4  $\mu\text{m}$  pore size would work. However, Taichman et al teach that CD34<sup>+</sup> hematopoietic bone marrow cells were seeded into the top chamber of TransWell dual-chambered 24-well plates with a 0.4  $\mu\text{m}$  pore size (e.g., page 1166, paragraph bridging columns). Thus, Taichman et al provide evidence that HSCs do not pass through 0.4  $\mu\text{m}$  filters, because the cells can be cultured on the surface of such filters. Further evidence that cells do not pass through 0.4  $\mu\text{m}$  filters is provided by US Patent Application Publication No. 2008/0085555 (e.g., paragraph [0045] teaches that pore sizes of 0.1 to 1  $\mu\text{m}$  do not allow the passage of cells), US Patent Application Publication No. 2005/0265978 (e.g., paragraph [0161], 0.4  $\mu\text{m}$  membrane prevents movement of cells), US Patent Application Publication No. 2010/0093077 (e.g., Figure 12, HSCs grow on 0.4  $\mu\text{m}$  filters), and US Patent Application Publication No. 2005/0181381 (e.g., paragraph [0196], both MSCs and HSCs grow on 0.4  $\mu\text{m}$  filters). Accordingly, one would not consider the arguments and evidence presented to be convincing with regard to the passage of haematopoietic cells through a plate base with pores of about 0.4 to 40 microns in diameter. One would not be able to practice the method with pore sizes from 0.1 to 1  $\mu\text{m}$  in diameter, because cells do not pass through these pores, and experimentation would be required to determine if other pore sizes, such as 2  $\mu\text{m}$  would work.

Applicant's arguments have been fully considered but are not deemed persuasive in view of the record as a whole. Therefore, the claims stand rejected under 35 U.S.C. 112, first paragraph.

***Conclusion***

No claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is (571)272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher Low can be reached on 571-272-0951. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications

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/Jennifer Dunston/  
Primary Examiner  
Art Unit 1636